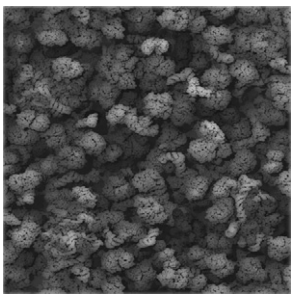
27-Subg
Protein Folding in Living Cells
Martin Gruebele.
U. of Illinois, Urbana, IL, USA.
Protein stability, folding and unfolding kinetics are examined in living mammalian and bacterial cells. Heterogeneous kinetics are seen in the nucleus, cytoplasm, ER, as well as among cells in a population. The diffusion coefficient for folding and tuning of the protein free energy landscape by the cellular milieu are quantified.

28-Subg
Crowding Induced Conformational Switch
Devarajan Thirumalai.
University of Maryland, College Park, MD, USA.
In this talk I will describe a general theoretical framework for describing conformational transitions in biomolecules and biomolecular complexes. The theory is based on the concept of depletion attraction well known in colloid science. The predictions of theory and simulations using coarse-grained models will be compared to specific experiments.

29-Subg
Protein Folding Trends in the Cellular Environment
Silvia Cavagnero.
University of Wisconsin-Madison, Madison, WI, USA.
The pathways of protein folding in vivo are still poorly understood despite the tremendous impact of correct folding for cell function. In the case of small/medium-size proteins, kinetic arguments suggest there is ample time for cotranslational conformational sampling, while nascent chains are still ribosome-bound and before synthesis of the full length protein is complete. Given the complexity of the cellular environment (molecular crowding, chaperones, ribosome), a combination of tailored biological and spectroscopic tools is likely needed to drive progress in this area. This lecture will present our current efforts to understand how proteins acquire the ability to attain their native state as nascent chains elongate from N to C terminus.

30-Subg
Challenges in Large-Scale Molecular Simulations of Intracellular Environments
Adrian H. Elcock.
University of Iowa, Iowa City, IA, USA.



Owing to advances in structural biology, proteomics and computational modeling it is now possible to consider constructing working simulation models of certain intracellular environments. Using bacterial systems for illustrative purposes, this talk will first briefly describe some promising results obtained with Brownian dynamics simulations methods - such as the ability to capture aspects of protein diffusion and thermodynamic stability in the bacterial cytoplasm. The talk will then discuss recent developments and ongoing projects before attempting to highlight a number of significant challenges likely to be faced by simulation models intended to more realistically describe events in vivo.

SUBGROUP: Permeation & Transport

31-Subg
Hydration Properties of Mechanosensitive Channel Pores: Dewetting and Stabilization of Conductive States
Sergei Sukharev.
University of Maryland, College Park, MD, USA.
MscS is the major osmolyte efflux valve that regulates turgor in many walled organisms from bacteria to higher plants. The crystal structure of WT *E. coli* MscS revealed a tight packing of seven pore-lining TM3 helices mediated by conserved Ala-Gly motifs. Complementarity of helical interfaces still leaves a 6.5Å unoccluded pore in the hydrophobic constriction (gate) formed by two rings of leucines. Initial computational studies suggested that that the gate is dehydrated and the crystal conformation should be non-conductive. Our further analysis indicated that the 'outer chamber' flanking the gate from the periplasmic side may also be dehydrated, producing a 'vapor plug' ~20Å long. Experimental analysis of hydrophilic substitutions in the outer chamber revealed decreases in the tension threshold and in-plane protein expansion on opening suggesting that the outer chamber is likely to be dehydrated at rest. The data reveal the principle difference between physical closure of the gate with the backbone-attached side chains and occlusion by vapor: water is mobile and after the breakage of the continuous phase it can freely search for new energy minima setting new boundaries and stabilizing nonconductive states. Under membrane tension of ~5-8 mN/m,MscS opens a 16Å pore that is fully hydrated. The opening transition begins with expansion of water-vapor interfaces, proceeding through wetting of the outer chamber and partial separation of TM3s, which ultimately leads to complete wetting of the hydrophobic pore. We discuss specifically the role of conserved glycines in TM3, which are buried in narrow closed conformations but exposed in the open states that critically changes the wetting characteristics of the pore lining from hydrophobic to hydrophilic and stabilize the conductive state of the channel. We discuss a similar open-state stabilizing mechanism in MscL.

32-Subg
Molecular Mechanisms of Ion Selectivity in Membrane Proteins: Ion Channels and Transporters
Sergei Noskov.
University of Calgary, Calgary, AB, Canada.
The mechanism of ion binding to membrane proteins and subsequent ion regulation of protein function is a subject that has fascinated scientists for a long time. Although the implications and applications of selective ion binding are many, ranging from management of cell volume to proper electric signaling upon subsequent transport across cellular membranes to being integral part of new nano-materials, our understanding of the underlying complex thermodynamics of ion binding and subsequent permeation across cellular membranes is very limited. Recent progress in structural studies of secondary amino-acid transporters provides us with a unique opportunity to address molecular mechanism of the cation selectivity in the protein with available high-resolution crystal structure and confirmed Na+/K+ or K+/Na+ selectivity. Combined results of our studies on K-selective channels (KcsA and MthK), non-selective channels (NaK) and Na+-coupled secondary transporters will be presented. A combination of the free energy simulations with classical and polarizable force-fields as well as recently developed QM/MM FEP protocol was used for evaluation of different factors in the observed selectivity in protein sites. Atomistics simulations blazed the trail for the development of an analytical theory than allows quantification of ion selectivity within a reduced framework. In conclusion, I will present a theory to clarify the molecular determinants of ion selectivity in protein binding sites developed recently (Yu et al., PNAS 2011) that integrates our current knowledge on monovalent cation selectivity.

33-Subg
Ion Selectivity and Permeation in a Potassium Ion Transporter
Ming Zhou.
Columbia University, New York, NY, USA.
The TrkH/TrkG/KtrB family of proteins mediate K^+ uptake in bacteria and likely evolved from simple K^+ channels by multiple gene duplications or fusions. We have solved the crystal structure of a TrkH from *Vibrio parahaemolyticus* to 3.5 Å. TrkH is a homodimer, and each protomer contains an ion permeation pathway. A selectivity filter, similar in architecture to that in K^+ channels but significantly shorter, is lined by backbone and side chain oxygen atoms. Functional studies showed that TrkH allows permeation of K^+ and Rb^+ but not smaller ions such as Na^+ or Li^+. Immediately intracellular to the selectivity filter is an intramembrane loop and an arginine residue, both highly conserved, that constrict an otherwise open permeation pathway. Functions of the loop and the positive charge (Arg) will be addressed.